110. (Amended) The isolated variant of claim 109, wherein the N413 mutation is N413D, and the V494 mutation is V494A.

Please add the following new claim:

112. (New) The isolated variant of any one of claims 63, 64, 83, 87, 91, 93, 95, 97, 99, 101, 103, 105, 107, and 109, having at least 60% amino acid sequence identity to the wild-type galactose oxidase, wherein the wild-type galactose oxidase has the sequence of SEQ ID NO:10 without the N537D mutation.

REMARKS

This submission is in response to the Final Official Action dated July 3, 2002. Claims 63-110 have been amended. New claim 112 has been added. Claim 111 has been allowed. Therefore, claims 63-112 are pending.

Claims 63-110 have been amended to recite a wild-type *D. dendroides* galactose oxidase. Support for this amendment can be found, e.g., on page 11, lines 1-4 and in Figs. 17A-C.

Claims 63, 64, 83, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109 have also been amended by indication of a percent amino acid sequence identity to a wild-type galactose oxidase. Support for this amendment can be found, e.g., on page 21, lines 3-7.

Claims 63 and 83 have also been amended to recite a mutation in an amino acid aligned with an amino acid of the wild-type galactose oxidase. This is supported at, e.g., page 21, lines 3-7.

Claims 84-110 have also been amended to recite improved D-galactose oxidation activity as compared to the wild-type galactose oxidase. Support for this amendment can be found throughout the specification, for example, on pages 47-48,

Table 4.

New claim 112 recites that the variants have at least 60% amino acid sequence identity to the wild-type galactose oxidase, and that the wild-type galactose oxidase has the sequence of SEQ ID NO:10 without the N537D mutation. Support for this claim can be found, e.g., at page 21, lines 3-7, and in Figs. 17A-C.

No new matter has been added by the amendments.

Reconsideration of the above identified application, in view of the above amendments and the following remarks, is respectfully requested.

Rejections Under 35 U.S.C. 112, 1st Paragraph

Written Description

Claims 63-110 stand rejected as allegedly not meeting the written description requirement. Specifically, the Examiner states that many structurally unrelated polypeptides are encompassed within the scope of these claims. The Examiner also contends that there is insufficient evidence of structure and functional correlation.

It is respectfully submitted that the pending claims, particularly as amended, are fully described by the specification.

All of the claims recite:

(1) that certain claimed variants having 60% sequence identity to the wild-type *D. dendroides* galactose oxidase are coupled with the additional requirement that specific mutations be present; and

(2) that certain claimed variants having 60% sequence identity to wild-type *D. dendroides* galactose oxidase are coupled with the additional requirement that specific mutations be present and that the claimed polynucleotide have improved D-galactose oxidation activity as compared to the wild-type galactose

oxidase.

The pending claims provide a genus of isolated polypeptides which are variants of a wild-type galactose oxidase and are structurally related in terms of sequence identity and shared mutations. These polypeptides have a structure defined in relation to a well-known wild-type galactose oxidase and its amino acid sequence, and specific mutations, which together provide a galactose oxidase function and/or improved galactose oxidase activity. The specification describes this structure-function relationship, in particular by providing specific mutations which result in improved galactose oxidation activity, and provides multiple galactose oxidase variants having improved enzymatic activity on the D-galactose substrate. For example, Table 4 shows the improved D-galactose oxidation activity of galactose oxidase variants, having the claimed mutations, as compared to the wild type.

Applicants respectfully invite the Examiner's attention to the revised Guidelines concerning compliance with the written description requirement. Attached hereto as Exhibit A is an excerpt of these Guidelines, describing an Example 14 similar to the presently claimed invention. The claim in Example 14 reads, "A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO:3 and catalyze the reaction of A --> B."

The analysis of the Example states, in part,

> The procedures for making variants of SEQ ID NO:3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO:3 wihch have 95% identity to SEQ ID NO:3 and retain its activity are conventional in the art. ... The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because fo the presence of an assay which applicant provided for identifying all of the at

least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity.

The Example concluded that the claim met the requirements for the written description requirement, even though no variant of SEQ ID NO:3 was actually present in the hypothetical specification.

In the context of the present invention, the pending claims are similar to, and more strongly supported than this Example from the Guidelines. Polypeptide function, reference sequence, and percentage sequence identity are all disclosed and claimed (see page 21, lines 4-9; pages 47-48, Table 4; and Figs. 17A-C).

The present claims also recite specific mutations and/or activity comparisons to that of wild-type galactose oxidase. Assay are provided for identifying improved galactose oxidase activity (see, e.g., Example 1 and Example 4).

Furthermore, the present specification describes numerous variant sequences that are functional as claimed, as opposed to the single sequence exemplified in the hypothetical. These examples are more than adequate to support the claimed genus. See *Regents of Univ. of California v. Eli Lilly*, 119 F.3d 1559, 1568 (Fed. Cir. 1997) ("[E]very species in a genus need not be described in order that a genus meet the written description requirement.").

Accordingly, as described and discussed above, the claims set forth isolated polypeptides of variants of a wild-type galactose oxidase by related functional and structural characteristics which fully comply with the written description requirement in particular when considering the Written Description Guidelines. It is respectfully submitted that this rejection should be reconsidered and withdrawn.

Enablement

Claims 63-110 stand rejected for allegedly not being enabled by the specification. Specifically, the Examiner concedes that the specification is enabling for mutant galactose oxidase from *D. dendroides*, but contends that the specification is not enabling for variants to other mutant galactose oxidases.

It is respectfully submitted that the amended claims are fully enabled by the specification. The amended claims provide that the isolated polypeptides encode for a variant galactose oxidase, are structurally related by having 60% sequence identity with wild-type *D. dendroides* galactose oxidase, have specific corresponding mutations, and exhibit a stated functional characteristic compared to the wild-type galactose oxidase. To the extent screening is necessary or desirable, the specification provides adequate guidance, and the work is routine and is not undue (see Examples 1 and 4).

The pending claims describe and enable isolated polypeptides encoding for a variant of a wild type galactose oxidase. While some experimentation may be required, no undue or unreasonable experimentation is required for one skilled in the art.

"To be enabling, the specification of the patent must teach those skilled in the art how to make and use the full scope of the claims of invention without 'undue experimentation.' " *Genentech Inc. v. Novo Nordisk, A/S*, 42 USPQ2d 1001, 1004 (Fed. Cir. 1997) (quoting *In re Wright*, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993)). The court has held that a patent specification complies with the statute even if a "reasonable" amount of routine experimentation is required but such experimentation must not be "undue". *Enzo Biochem Inc. v. Calgene*, Inc. 52 USPQ2d 1129, 1135 (Fed. Cir. 1999) (citing *In re Wands*, 8 USPQ 2d at 1404). The court in *Enzo Biochem* looked favorably on the factors set forth in *In re Wands* to consider in determining

whether disclosure requires undue experimentation, which are:

(1) the quantity of experimentation necessary,

(2) the amount of direction of guidance presented,

(3) the presence or absence of working examples,

(4) the nature of the invention,

(5) the state of the prior art,

(6) the relative skill of those in the art,

(7) the predictability or unpredictability of the art, and

(8) the breadth of the claims.

Enzo Biochem, 52 USPQ 2d at 1135-1136 (quoting *In re Wands*, 8 USPQ 2d at 1404).

Applying the Wands factors to the facts set forth above to the amended claims shows that the specification enables the claimed invention.

First, the quantity of experimentation is not excessive. Contrary to the Examiner, it is routine in this field to screen large numbers of sequences for common properties, such as enzyme activity, particularly if the sequences are derived from a common parent or have a common evolutionary origin. It is routine for one skilled in the art to screen for the specifically desired variants, having 60% sequence identity to the sequence provided as a wild-type galactose oxidase. As in Example 14 of the Guidelines, assay procedures for this purpose are provided (see Examples 1 and 4). Notably, Example 1 (page 31, lines 28-30) recites: "This simple method has suitable sensitivity and can be used to evaluate several thousands colonies on one membrane at once", thus supporting that the mutants of the invention, having improved D-galactose activity, can be identified by efficient high-throughput screening methods.

Second, the specification provides more than ample guidance for practicing the invention, including the high-throughput screening methods and

sequence alignment methods, as well as the requisite sequence identity with specific mutations or desired activity (see page 21 and Examples 1 and 4).

Third, working examples for preparing, screening for, and identifying the variants of a wild-type galactose oxidases are fully described in Examples 1-3.

Fourth, practitioners recognize that there is unpredictability in the field of genetic engineering, and consider screening techniques like those disclosed in the specification to be routine and reasonable discriminatory tools. Here, there is specific guidance for using these tools to practice the claimed invention (see Examples 1 and 4).

With respect to the fifth and sixth points of the state of the prior art and the level of the skill in the art, the prior art provides techniques for the sequence alignment of a galactose oxidase and identification of corresponding residues, and the level of skill in the art is relatively high.

Seventh, although there may be unpredictability in the creation of protein variants using directed evolution, the directed evolution and high-throughput methods described herein enable the creation and screening of a large number of variants with a reasonable expectation of success. The relative ease and speed of such methods, and their benefits, are well recognized in the field. See, e.g., the Whittaker publication, enclosed as Exhibit A with the Response to Official Action filed April 15, 2002.

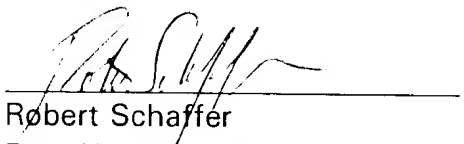
Finally, the amended claims describe and claim both structural and functional features of the variants of galactose oxidase enzymes of the present invention, and are not unduly broad.

Accordingly, the invention as set forth by the amended claims is fully enabled. Reconsideration and withdrawal of this rejection is respectfully requested.

Therefore, in view of the above amendments and remarks, it is respectfully requested that the application be reconsidered and that all pending claims be allowed and the case passed to issue.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



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Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

EXPRESS MAIL CERTIFICATE

Date 12/2/02 Label No. EV 085547257 US

I hereby certify that, on the date indicated above, this paper or fee was deposited with the U.S. Postal Service & that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, DC 20231 by "Express Mail Post Office to Addressee" service.

JB Pak

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Arnold, **FRANCIS**; Petrounia, **IONNA**; Sun, **LIANHONG**

Serial No.: 09/722,602 Art Unit: 1652

Confirmation No.: 5781

Filed: 11/27/00 Examiner: Y. Pak

For: **DIRECTED EVOLUTION OF OXIDASE ENZYMES**

MARK UP ACCOMPANYING RESPONSE TO OFFICIAL ACTION

63. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in at least one amino acid [corresponding to] aligned with an amino acid selected from the group consisting of A3, S10, M70, P136, G195, T218, L312, V494, C515, N535, N537, [and] S610 [of SEQ ID NO:18 and], N413 and S550 of [SEQ ID NO:10] the wild-type galactose oxidase.

64. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides*

galactose oxidase and at least one of the amino acid mutations corresponding to S10P, M70V, G195E, V494A, C515S, N535D, [and] N537D [of SEQ ID NO:18] and N413D of [SEQ ID NO:10] the wild-type galactose oxidase.

65. (Twice amended) The isolated [galactose oxidase] variant of claim 64, which has the amino acid mutation corresponding to N537D of [SEQ ID NO:18] the wild-type galactose oxidase.

66. (Twice amended) The isolated [galactose oxidase] variant of claim 64, which has the amino acid mutation corresponding to V494A of [SEQ ID NO;18] the wild-type galactose oxidase.

67. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising the amino acid mutation corresponding to C515S of [SEQ ID NO:18] the wild-type galactose oxidase.

68. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising the amino acid mutation corresponding to S10P of [SEQ ID NO:18] the wild-type galactose oxidase.

69. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising a silent mutation at a position corresponding to P136 of [SEQ ID NO:18] the wild-type galactose oxidase.

70. (Twice amended) The isolated [galactose oxidase] variant of claim 68, further comprising a silent mutation at a position corresponding to P136 of [SEQ ID NO:18] the wild-type galactose oxidase.

71. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising the amino acid mutation corresponding to G195E of [SEQ ID NO:18] the wild-type galactose oxidase.

72. (Twice amended) The isolated [galactose oxidase] variant of claim 71, further comprising a silent mutation in at least one of positions corresponding to A3 and P136 of [SEQ ID NO:18] the wild-type galactose oxidase.

73. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising the amino acid mutation corresponding to N535D of [SEQ ID NO:18] the wild-type galactose oxidase.

74. (Twice amended) The isolated [galactose oxidase] variant of claim 73, further comprising a silent mutation in at least one of positions corresponding to P136, L312, and T218 of [SEQ ID NO:18] the wild-type galactose oxidase.

75. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising the amino acid mutation corresponding to M70V of [SEQ ID NO:18] the wild-type galactose oxidase.

76. (Twice amended) The isolated [galactose oxidase] variant of claim 75, further comprising a silent mutation at a position corresponding to P136 of [SEQ ID NO:18] the wild-type galactose oxidase.

77. (Twice amended) The isolated [galactose oxidase] variant of claim 64, which has the amino acid mutations corresponding to S10P, M70V, G195E, V494A and N535D of [SEQ ID NO:18] the wild-type galactose oxidase.

78. (Twice amended) The isolated [galactose oxidase] variant of claim 77, further comprising a silent mutation at a position corresponding to P136 of [SEQ ID NO:18] the wild-type galactose oxidase.

79. (Twice amended) The isolated [galactose oxidase] variant of claim 64, which has the amino acid mutation corresponding to N413D of [SEQ ID NO:10] the wild-type galactose oxidase.

80. (Three times amended) The isolated [galactose oxidase] variant of claim 79, further comprising a silent mutation at a position corresponding to S550 of [SEQ ID NO:10] the wild-type galactose oxidase.

81. (Twice amended) The isolated [galactose oxidase] variant of claim 66, further comprising the amino acid mutation corresponding to N413D [SEQ ID NO:10] of the wild-type galactose oxidase.

82. (Twice amended) The isolated [galactose oxidase] variant of claim 81, further comprising a silent mutation in at least one of a position corresponding to S550 and S610 of [SEQ ID NO:10] the wild-type galactose oxidase.

83. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in at least one amino acid [corresponding to a position] aligned with an amino acid selected from the group consisting of A3, S10, M70, P136, T218, L312, C515, N535, N537, S550, [and] S610 [of SEQ ID NO:18], and N413 of [SEQ ID NO:10] the wild-type galactose oxidase.

84. (Twice amended) The isolated [galactose oxidase] variant of claim 83, further comprising at least one amino acid mutation corresponding to a mutation selected from the group consisting of G195 and V494 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

85. (Twice amended) The isolated [galactose oxidase] variant of claim 83, wherein the mutation is selected from a mutation corresponding to at least one of the group consisting of S10P, M70V, N413D, C515S, N535D, and N537D of [SEQ ID NO:18 and N413D of SEQ ID NO:10] the wild-type galactose oxidase.

86. (Twice amended) The isolated [galactose oxidase] variant of claim 85, further comprising at least one amino acid mutation corresponding to a mutation selected from the group consisting of G195E and V494A of [SEQ ID NO:18] the wild-type galactose oxidase.

87. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in an amino acid corresponding to N537 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

88. (Twice amended) The isolated [galactose oxidase] variant of claim 87, wherein the mutation is N537D.

89. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides*

galactose oxidase and mutations in amino acids corresponding to V494 and C515 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

90. (Twice amended) The isolated [galactose oxidase] variant of claim 89, wherein the mutations are V494A and C515S.

91. (Amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type galactose oxidase from *D. dendroides* and mutations in amino acids corresponding to V494 and P136 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

92. (Twice amended) The isolated [galactose oxidase] variant of claim 91, wherein the V494 mutation is V494A.

93. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, and S10 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

94. (Twice amended) The isolated [galactose oxidase] variant of claim 93, wherein the V494 mutation is V494A, and the S10 mutation is S10P.

95. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, G195, and A3 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

96. (Twice amended) The isolated [galactose oxidase] variant of claim 95, wherein the V494 mutation is V494A, and the G195 mutation is G195E.

97. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, L312, N535, and T218 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

98. (Twice amended) The isolated [galactose oxidase] variant of claim 97, wherein the V494 mutation is V494A, and the N535 mutation is N535D.

99. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, and M70 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

100. (Twice amended) The isolated [galactose oxidase] variant of claim 99, wherein the V494 mutation is V494A, and the M70 mutation is M70V.

101. (Twice amended) An isolated galactose oxidase [which has] variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, S10, P136, M70, G195, and N535 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

102. (Twice amended) The isolated [galactose oxidase] variant of claim 101, wherein the V494 mutation is V494A, the S10 mutation is S10P, the M70 mutation is M70V, the G195 mutation is G195E, and the N535 mutation is N535D.

103. (Amended) An isolated galactose oxidase [which has]variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in an amino acid corresponding to N413 of [SEQ ID NO:10] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

104. (Twice amended) The isolated [galactose oxidase] variant of claim 103, wherein the mutation is N413D.

105. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase [which has] and a mutation in amino acids corresponding to N413 and S550 of [SEQ ID NO:10] the wild-type galactose oxidase, and wherein the variant has

improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

106. (Twice amended) The isolated [galactose oxidase] variant of claim 105, wherein the N413 mutation is N413D.

107. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase [which has] and a mutation in amino acids corresponding to N413 [of SEQ ID NO:10, and] S550 and V494 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

108. (Twice amended) The isolated [galactose oxidase] variant of claim 107, wherein the N413 mutation is N413D, and the V494 mutation is V494A.

109. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase [which has] and mutations in amino acids corresponding to N413 [of SEQ ID NO:10, and] S550, V494, and S610 of [SEQ ID NO:18] the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

110. (Amended) The isolated [galactose oxidase] variant of claim 109, wherein the N413 mutation is N413D, and the V494 mutation is V494A.